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Multienzyme decorated polysaccharide amplified electrogenerated chemiluminescence biosensor for cytosensing and cell surface carbohydrate profiling

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ABSTRACT

A novel ECL biosensor for cytosensing and cell surface carbohydrate expression evaluation was developed, by the integration of the peptide modified interface for highly specific carbohydrate recognition and sodium alginate loaded glucose oxidase as the signal probe with high signal amplification efficiency. A cysteine-terminated peptide self-assembled on the electrode through Au–S bond to construct a functional interface for cell capture, with decent biocompatibility and high affinity for the human breast cancer cell MCF-7. Concanavalin A lectin modified gold nanoparticles specifically recognized the cell surface carbohydrates and were absorbed on the electrode, followed by the immobilization of multiple glucose oxidase conjugated sodium alginate, which could remarkably increase the sensitivity of the biosensor with enhanced catalysis. The as-proposed ECL cytosensor was successfully applied for the detection of the MCF-7 tumor cells, whose glycans on the cell membranes are over-expressed. A low detection limit of 150 cells mL^{-1} was obtained, with a wide dynamic linear range from 5.0×10^{2} to 5.0×10^{5} cells mL^{-1}. Due to the excellent sensitivity, stability and biocompatibility, the ECL biosensor would be promising in reliable diagnostics of glycan relevant biomarkers for cancer and other diseases.

1. Introduction

Cells are covered by a number of glycans which are covalently bound to the underlying proteins or lipids with high density and complex diversity (Linhardt and Toshihiko, 2004). The carbohydrates on the cell membrane possess abundant information for specific molecular recognition and play important roles for cell migration, proliferation and communication (Dennis et al., 2009; Hirabayashi, 2008). Its abnormal expression has been proved to be related with many diseases, especially Alzheimer's disease and cancers (Rexach et al., 2008; Zhao et al., 2008). Therefore, developing glyco-biosensors for synchronously cytosensing and cell surface carbohydrate expression analysis is significant in clinical diagnostics. It will provide a platform to understand the roles of carbohydrates in disease progression and monitor the glycan relevant biomarkers. Recently, a serious of approaches have been developed for probing carbohydrate-protein interactions, cell surface carbohydrates profiling and cytosensing, including electrochemical impedance spectroscopy (Hu et al., 2013), quartz crystal microbalance (Shen et al., 2007), fluorescence polarization (Kakehi et al., 2001), localized surface plasmon resonance (Bellapadrona et al., 2012), and field effect transistors (Vedala et al., 2011). However, the sensitivity and stability in most of these methods are still limited, because the modifications on the biosensing interfaces induce the less active accessibility and weak affinity between monosaccharides and lectin as well as the loss of biocompatibility. Fluorescent assays also have drawbacks such as false positives, high background and complicated labelling procedures. Thus, it is still a critical demand on simple, rapid, and low-cost detection technologies for the accurate and sensitive analysis of cancer cells and profiling of cell surface carbohydrates.

To avoid the loss of biocompatibility and weak recognition affinity, some peptides have been used for targeting different tumors and cell types (Zhang et al., 2001; Stangl et al., 2014). Among these, tumor homing peptides RGD could target the $\alpha_v\beta3$ integrin in the tumor cells and NGR could recognize aminopeptidase N receptors in the vasculature (Zitzmann et al., 2002; Corti et al., 2008). Besides, peptides arrays formed by short peptides covalently bound to a solid surface showed specific binding affinity to the cells (Veiseh et al., 2007).

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Combining with their high specificity, strong binding force and biocompatibility, peptides show brilliant prospect for developing sensing interface for the cancer cells.

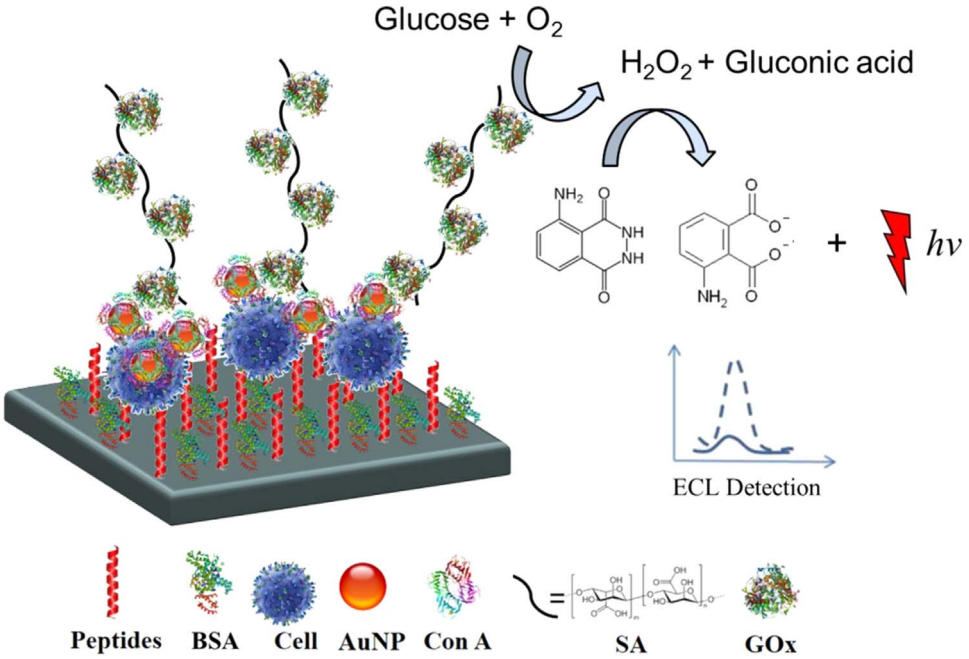
Electrogenerated chemiluminescence (ECL) technique, which involves electron-transfer reactions and light-emitting process of the luminophores on the electrode (Richter, 2004; Marquette and Blum, 2009), is a powerful analytical tool with advantages of low cost, low background noise, wide dynamic concentration response range and high sensitivity (Liu et al., 2015). It has been widely applied in small molecule detection (Wang et al., 2009), immunoassay (Xu et al., 2011), cell analysis (Han et al., 2011) and clinical diagnosis (Zhang et al., 2012). Meanwhile, on account of the good biocompatibility, fascinating electrocatalytic activity, large surface area, and excellent conductivity, nanomaterials such as gold nanoparticles (Li et al., 2016), quantum dots (Li and Zhu, 2013), metal oxide nanorods (Lu et al., 2006), carbon nanotubes (Zhang et al., 2004) and graphene (Zhang et al., 2014) have been widely used in electrochemistry, biosensing and imaging. The unique properties of nanomaterials allow them to be interfaced with biomolecules and open up a wide range of biological applications (Cha et al., 2013; Wang et al., 2014a, 2014b; Guo et al., 2014). Nanomaterials can also function as efficient carriers for multiple carbohydrate presentation to achieve sufficiently high affinities required for sensitive recognition and labels for signal amplification. (Wang et al., 2003; Zhang et al., 2007).

In this work, a sensitive ECL biosensor, using peptides as capture probes, Concanavalin A (Con A) modified gold nanoparticles (Con A@AuNPs) as multiple-site combined probes and glucose oxidase conjugated sodium alginate (GOx@SA) as nanoprobes, was developed for selectively cancer cell detection and cell surface carbohydrate expression evaluation (Scheme 1). To demonstrate the principle, a specific peptide for human breast cancer cell MCF-7 cell was chosen as capture probe. Glucose oxidase, an important mannose-containing glycoprotein (Wilson and Turner, 1992), was used to catalyze the oxidation of glucose to produce hydrogen peroxide which significantly improves the ECL signal of luminol (Wang et al., 2013). Con A, which has specific affinities to the sodium alginate, the mannose on GOx, and glycan on the cell surface, served as the recognizer for cell surface carbohydrates and the capturer for GOx@SA nanoprobes. Sodium alginate, a kind of natural polysaccharide composed by L-mannuronic acid and D-guluronic acid at a certain proportion, which could interact with Concanavalin A (Con A) (Holme et al., 2008), was used as the carrier to load multiple GOx with plenty of carboxylic acid on its structure, to form the signal probe. Owing to its good hydrophily, low adhesion and excellent biocompatibility, sodium alginate could fix the enzymes without damaging their structure and bioactivity (Prang et al., 2006). Moreover, sodium alginate provides much more recognition sites for the conjugation of biomolecules and dramatically improves the sensitivity and selectivity of the biosensor. The experimental observations indicate that this strategy can not only recognize the cells with high specificity but also detect the cell and its surface glycan with high sensitivity. Such a simple and sensitive biosensor would be promising in reliable diagnostics of glycan relevant biomarkers for cancer and other diseases.

2. Experimental section

2.1. Materials and reagents

Sodium Alginate (SA) and Coomassie Brilliant Blue G250 (G250) were provided by Aladdin (China). Bovine serum albumin (BSA) was purchased from Dingguo Biological Products Company (China). Glucose oxidase (GOx, 100–250 units per mg) was obtained from Sangon Biotech (China). Concanavalin A (Con A) and luminol were received from Sigma-Aldrich (USA). $HAuCl_4{\cdot}3H_2O$ (48% w/w) was obtained from Shanghai Regent (China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) were provided by Alfa Aesar (USA). Cysteine-terminated peptide WC10 (WLEAAYQRFLC, 98%) was purchased from GL Biochem (China). Other reagents of analytical grade were obtained from Sinopharm Chemical Reagent Company (China) and were used as received. 100 mM Phosphate buffer solutions (PBS) were prepared with $Na_2HPO_4{\cdot}12H_2O$ and $NaH_2PO_4{\cdot}2H_2O$, containing 137 mM NaCl



Scheme 1. Configuration of ECL biosensor for selective detection of cancer cell and cell surface carbohydrate profiling. The cysteine-terminated peptide is firstly immobilized on the gold electrode through the Au-S bond for specific recognition of cancer cell MCF-7. After cell capturing, BSA is used to block the electrode to avoid nonspecific adsorption. Then, the Con A lectin conjugated gold nanoparticles (Con A@Au NPs) are absorbed on the cell surface due to the affinity between Con A and glycans. Thereafter, the GOx modified sodium alginate (GOx@SA) is immobilized via the affinities between the oligosaccharides on sodium alginate/GOx and Con A lectins on Au NPs, which catalyzes the oxidation of glucose to produce hydrogen peroxide and significantly improve the ECL signal of luminol.